A prospective comparison of fine-needle aspiration cytology and histopathology in the diagnosis and classification of lymphomas

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Introduction: Surgical biopsy examination is the gold standard for lymphoma diagnostics. However, fine-needle aspiration cytology (FNAC) offers several advantages in that it is quick, inexpensive, and the aspiration procedure has very few complications. This prospective study compares the diagnostic outcome between FNAC and surgical biopsy.
Materials and methods: A total of 103 patients (>15 years) with lymphadenopathy and accessible lymph nodes underwent both diagnostic procedures. Immunophenotyping was performed on both FNAC and histopathological specimens. The updated KIEL classification was used for primary diagnosis and the WHO classification for reclassification.
Results: FNAC- and histopathology-based diagnoses were concordant in 76 patients. In 10 patients, there was a major diagnostic discordance: four differed with regard to degree of malignancy (low- versus high-grade NHL), three lymphoma versus reactive changes, and three regarding Hodgkin's lymphoma versus anaplastic large cell lymphoma. In 10 patients there was some (minor) discordance regarding subclassification: in eight patients the results of immunophenotyping differed, in two cases there were discrepancies in the cell type classification. In the remaining seven cases, there were diagnostic difficulties due to an insufficient sample. two serious adverse events occurred following surgical biopsy.
Conclusions: FNAC is an accurate method in the diagnosis of lymphomas when the cytologic diagnosis is corroborated by immunophenotyping. However, an increasing use of FNAC for primary diagnosis and classification of lymphomas may result in a loss of archival tissue for complementary analyses, reclassification, and research purposes. In addition, some of the lymphoma entities are impossible to diagnose with use of the FNAC technique.
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Introduction

Histological examination of lymphoid tissue is traditionally considered the gold standard for lymphoma diagnosis. Histopathology has documented advantages for the classification of lymphomas, providing material for complementary analyses, and for clinical research purposes.[1,2] Also, the widely used lymphoma classification systems such as KIEL and Working Formulation were based on histopathology.[3,4] The REAL classification and the new WHO lymphoma classification combine morphology with immunophenotype, and genetic and clinical features.[1,5–8] Fine-needle aspiration cytology (FNAC) offers immediate preliminary diagnosis in the investigation of lymphadenopathy with minimal trauma to the patient at a considerably lower cost than surgical biopsy.[9–11] The method of aspiration cytology began to receive international attention as early as 1947 after publications by Zajicek, Franzen, Esposti, and Löwhagen at the Karolinska Hospital, Stockholm.[12] Thus, due to the long tradition, a large number of patients with lymphadenopathy have been investigated by FNAC at our hospital. At other centers, until recently, FNAC has been predominantly used for lymphoma staging and to confirm recurrent or residual disease.[10,11,13] Nowadays, FNAC has gained acceptance as a diagnostic tool in certain risk patients and is also establishing a more general role in the primary diagnosis

and subclassification of lymphomas.[9,10,14,15] This is mainly due to the application of ancillary investigations, which increase the accuracy of diagnosis when compared to cytological examination alone.[9,16,17] FNAC has also been advocated as a useful method in comparison to more expensive surgical excision biopsies in developing countries with limited financial and health-care resources.[18] However, published prospective studies evaluating the reliability of FNAC in comparison to histopathology in patients with lymphoma remain rare. The aim of this study was to assess the clinical usefulness of FNAC in a prospective cohort of patients suspected of having lymphoma.

Patients and methods

Study design

Eligible patients were men and women (>15 years) with lymphadenopathy and accessible lymph nodes. Tissue samples from the same location were consecutively studied first by FNAC and, later, if lymphoma was suspected, by histological examination of a biopsy from the same lymph node site within a pre-defined time interval of less than 2 months (Table 1). Patients with a FNAC result suggesting reactive lymphoid hyperplasia did not undergo surgical biopsy and were not included in the final study cohort. Patients with lymphadenopathy due to metastatic carcinoma were also excluded from the analysis. The protocol of this prospective study was reviewed and approved by the Ethics Committee at the Karolinska Hospital.

FNAC

All percutaneous fine-needle aspiration (FNA) biopsies were performed with a 0.6-mm needle according to the procedure described by Zajicek.[19] One part of the aspirate was used to prepare smears, which were air-dried and stained by May–Grünwald–Giemsa (MGG) or methanol-fixed and stained by the Papanicolaou technique. Additional air-dried smears were fixed in freshly prepared buffered 4% formalin and used for proliferation marker (MIB-1) immunostaining. The second part of the aspirate was suspended in phosphate-buffered saline (PBS) and used to make cytospin (Shandon, Cheshire, UK) preparations for immunocy-

tochemical analysis, as previously described.[9] Adequacy of cell viability and cell concentration in suspensions was assessed immediately after the FNAC procedure.[9] The cytologic diagnosis was made by two experienced cytopathologists (ET and LS) and based on the updated KIEL classification.[3] Details of immunocytochemistry, antibody panel, and growth fraction analyses have been described previously.[9,20–23] In short, three-step alkaline phosphatase immunostaining was employed for the immunologic characterization of all cases. Proliferation fraction analysis was performed using antibody Ki-67 (MIB-1) (Immunotech, Marseille, France) detected by the immunoperoxidase–avidin–biotin complex method. The percentage of proliferating tumor cells was determined by counting at least 200 neoplastic cells at a high-power field magnification in randomly selected areas of the smears.

Biopsy material

The representative fragments of excised lymph nodes were fixed in formalin or B5 fixative and routinely processed. Hematoxylin and eosin (HE), periodic-acid-Schiff (PAS), Giemsa and Gordon-Sweet stains were performed on 6 μm paraffin sections. Cell suspensions were prepared from fragments of the biopsies and the immunophenotyping was performed by flow cytometry with double direct immunofluorescence staining using antibodies to CD19, CD20, CD22, CD10, CD5, CD3, CD4, CD8, kappa and lambda Ig light chains (from Becton & Dickinson, Stockholm, Sweden, or Dakopatts, Glostrup, Denmark). In cases where the biopsies were fixed immediately after excision, the immunophenotyping was performed on paraffin sections by standard immunoperoxidase method (described in detail in Axdorph et al.[24]). The initial histopathologic diagnosis was made by experienced hematopathologists (Åö and/or APM) and based on the updated KIEL classification.[3] All cases with minor or major (see below) diagnostic discrepancies between FNAC and histopathology were reclassified by the same observers and external reviewers according to the updated REAL and WHO classifications.[5,6]

Data analysis

Discordant cases were identified by comparing the diagnostic results achieved by FNAC versus histo-

Table 1 Distribution of lymphoma subtypes diagnosed by histopathology and FNAC (updated KIEL classification; number of patients)

	CLL	IC	PC	CB/CC	CC	CB	IB	TCR	AILD	MZL	Mantle	Ki-1	LB	HML	HL	R	Total
Patients who underwent FNA and/or biopsy for histopathology at Karolinska Hospital during the study period																	
Hist	6	26	1	48	9	23	3	1	2	1	1	4	2	10	49	33	224
FNAC	17	44	1	60	1	45	11	1	0	2	0	8	15	10	37	368	620
Patients in the study																	
Hist	3	10	1	24	2	16	2	0	0	0	0	2	0	7	32	4	103
FNAC	3	13	1	27	1	13	3	1	0	1	0	3	2	3	31	1	103

Hist = histopathology; CLL = chronic lymphocytic leukemia; IC = immunocytoma; PC = plasmacytoma; CB/CC = centroblastic/centrocytic; CC = centrocytic; CB = centroblastic; IB = immunoblastic; TCR = T-cell rich B cell; AILD = angioimmunoblastic; MZL = marginal zone lymphoma; LB = lymphoblastic; HML = high malignant lymphoma; HL = Hodgkin's lymphoma' R = reactive lymph node.

pathology. These were divided into two groups: major diagnostic discordance with potential clinical relevance (Table 2), or minor diagnostic discordance without clinical relevance. For all patients with a major diagnostic discordance, special attention was focused on clinical status at diagnosis, choice of first-line therapy, and response to treatment (Table 2). Treatment decisions were made at the discretion of the responsible clinician and recorded retrospectively.

Adverse events

In keeping with international guidelines,[25] an adverse event was defined as any undesirable event, which occurred while the patient underwent FNA and/or surgical biopsy for histopathology. The occurrence of adverse events was assessed in 94 of the included patients (91%).

Statistics

Conventional descriptive statistical methods and χ^2 test were used.

Results

Between October 1990 and February 1993, the total numbers of patients with lymphadenopathy who underwent FNAC and/or surgical biopsy for histopathology at Karolinska Hospital were 620 and 224, respectively (Figure 1). Out of 620 FNAC study samples, 368 (59%) showed non-lymphoma conditions, for example, reactive lymphadenopathy, cancer metastasis, and tuberculosis. In these patients an open biopsy was not performed. Another 96 (15%) of patients studied by

FNAC were not subjected to surgical biopsy for the following reasons: rapidly progressive disease with intermediate need for treatment, no easily accessible lymph nodes, and elderly patients with a poor performance status. Of these 96 patients, 24 (25%) were diagnosed with high-grade NHL. During the study period, 48 patients were subjected to surgical biopsy without preceding FNA. These patients were referred to us from other departments, for example, Ear, Nose, and Throat Department. Thus, 176 patients underwent both diagnostic procedures. In all, 73 patients (41%) were excluded due to either a nonmatching site of biopsy or too long of a time interval between the two procedures (>2 months; Figure 1). Among the remaining 103 patients, there were 47 females and 56 males (median age 62 years; range 23–85). The distribution of lymphoma types did not differ between the study group and all lymphoma patients diagnosed at Karolinska Hospital during the study period ($P > 0.05$ χ^2 test; Table 1).

Evaluation of diagnostic methods

The diagnoses were concordant in 76 patients (74%). In 10 patients (10%), a major discordance between FNAC and histopathologic diagnoses was found (Table 2). These patients are presented in detail below. In the remaining 17 patients, minor discordances were recorded due to discrepancy in immunophenotyping results ($n = 8$), insufficient material ($n = 7$) or differences in the cell classification between FNAC and histopathology ($n = 2$). In the group with discrepancies in the immunophenotyping results, a monoclonal expression of Ig light chains was detected by immunocytochemistry on cytospins in seven cases. These results could not be

Table 2 Clinical characteristics of 10 patients with major diagnostic discordance

Pt no.	Age (years)	Sex	Clinical stage	FNAC diagnosis	Histopath diagnosis[a]	Reviewed histopath. diagnosis[b]	Treatment	Response to therapy	Outcome
1	39	M	IA	MZL	Reactive lgl	Reactive lgl	RT	CR	Relapse, local RT, CCR
2	60	F	IA	CB/CC	Reactive lgl	Reactive lgl	RT	CR	CCR
3	72	F	IIIA	CB/CC	High-grade B-NHL	DLBL	RT, CT (CHOP)	PR	PD, deceased
4	68	M	IVB	CB/CC[c]	CB/CC	FL	CT (CHOP)	PD	Deceased
5	70	F	IVA	Ki-1	HL NS	HL NS	CT (CHOP)	PD	Deceased
6	27	M	IA	Ki-1	HL NS	HL NS	CT (CHOP)	CR	CCR
7	30	M	IVB	HD	Ki-1	ALCL	RT, CT (MOPP/ABVD)	CR	CCR
8	83	M	IVB	CB/CC	CB	DLBL	Palliation	PD	Prostate cancer, deceased
9	64	M	IIIA	IC III	Reactive lgl	Reactive lgl	CT (CHOP)	PR	Adenocarcinoma, deceased
10	59	F	IA	CB/CC	CB	DLBL	RT	CR	Relapse, CHOP chemotherapy

[a]Updated KIEL classification. [b]WHO classification. [c]In transformation.
CR = complete remission; PR = partial remission; PD = progressive disease; CCR = continuous complete remission; RT = radiotherapy; CT = chemotherapy; MZL = marginal zone lymphoma; CB/CC = centroblastic/centrocytic; DLBL = diffuse large B-cell lymphoma; FL = follicular lymphoma; HL NS = Hodgkin's lymphoma nodular sclerosis; ALCL = anaplastic large cell lymphoma; CB = centroblastic; IC III = polymorphous immunocytoma.
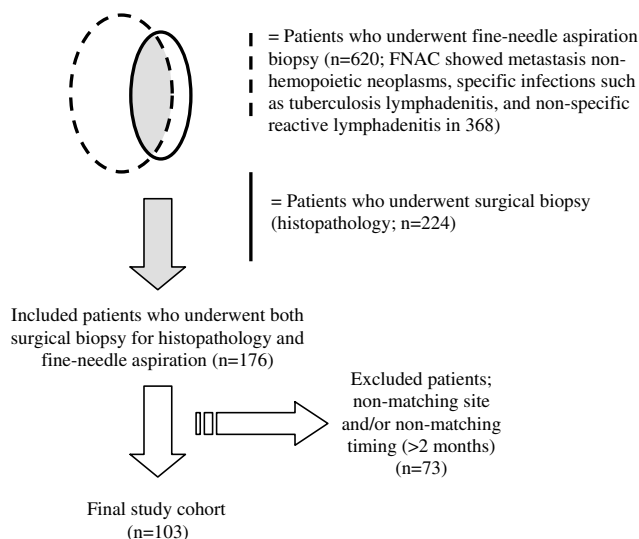
= Patients who underwent fine-needle aspiration biopsy (n=620; FNAC showed metastasis non-hemopoietic neoplasms, specific infections such as tuberculosis lymphadenitis, and non-specific reactive lymphadenitis in 368)

= Patients who underwent surgical biopsy (histopathology; n=224)

Included patients who underwent both surgical biopsy for histopathology and fine-needle aspiration (n=176)

Excluded patients; non-matching site and/or non-matching timing (>2 months) (n=73)

Final study cohort (n=103)

Figure 1 Algorithm of patients undergoing FNA and/or surgical lymph node biopsy.

confirmed on paraffin sections, probably due to technical reasons, for example the prolonged fixation. In three of these patients, flow cytometric immunophenotyping was also performed and light chain restriction was not detected. In one patient, the results of flow cytometric immunophenotyping of lymph node cell suspensions indicated that lymphoma cells were weakly positive for CD5, which was not detected by immunocytochemistry on cytospin. One of the two patients with differences in the cell classification had centroblastic lymphoma according to histopathology, while the FNAC diagnosis was immunoblastic lymphoma. In the other patient, a histopathological diagnosis of centroblastic lymphoma was established, but according to FNAC the diagnosis was a lymphoblastic lymphoma. At the re-evaluation (REAL/WHO) both these biopsies were classified as diffuse large B-cell lymphoma (DLBL).

Clinical impact

At the time of decision of treatment, the responsible clinician had access to both the FNAC and the histopathologic diagnoses, including immunophenotyping results. In 10 patients, major discordance in diagnosis was found as described below (Table 2):

Patient No. 1 had a 6-year-history of Sjögren's syndrome with local lymphadenopathy without evidence of lymphoma. At entry into our study, the biopsy showed reactive findings, but according to FNAC the patient had a marginal zone lymphoma. The patient had stage I disease and remained in clinical remission for 4 years following local radiotherapy. At this time, a lymphoma in his left eye lid was diagnosed and again radiotherapy was given. Patient No. 2 had reactive changes according to histopathology and a follicular lymphoma (FL [WHO] or CB/CC [Kiel]) according to

FNAC. The patient was not treated primarily but given local radiotherapy 2.5 years later when histopathology confirmed the FC lymphoma diagnosis in another lymph node. The patient remains in clinical remission 5 years after treatment. According to histopathology Patient No. 3 had high-grade B-NHL (KIEL) or DLBL (WHO), while the FNAC diagnosis was follicular lymphoma (CB/CC) with a high proliferation rate (30%). Patient No. 4 was diagnosed with FL (CB/CC) by histopathology and FL in transformation (proliferation rate 50%) according to FNAC. In two patients (Nos 5 and 6), the diagnosis according to FNAC was anaplastic large cell lymphoma (ALCL or Ki-1 NHL) but after the biopsy a histopathological diagnosis of Hodgkin's lymphoma nodular sclerosis (HL NS) was established. Both patients received CHOP treatment.[26] According to histopathology, Patient No. 7 had a Ki-1 NHL (anaplastic large cell lymphoma; ALCL), while FNAC showed HL unclassified. The patient received MOPP/ABVD[27] chemotherapy followed by local radiotherapy. In Patient No. 8, the FNAC diagnosis was FL (CB/CC) with relatively high proliferation rate (30%). The biopsy examination revealed DLBL (WHO) (centroblastic [CB] NHL, Kiel). Owing to the high age of the patient and severe comorbidity, only palliative treatment was given. Patient No. 9 was given CHOP chemotherapy for CB (DLBL) and remained in clinical remission for 6 years before inclusion in this study. When included in the study, the FNAC diagnosis was polymorphous immunocytoma and histopathology showed a reactive lymph node. The patient was given one course of MIME chemotherapy.[28] Unfortunately, the patient developed a cerebral hemorrhage and his clinical condition did not allow any further treatment. The patient eventually died and autopsy revealed spread metastases of undifferentiated adenocarcinoma, but there were no signs of malignant lymphoma. Patient No. 10 was successfully treated by local radiotherapy for a DLBL (CB) and was in clinical remission 10 years before inclusion in this study. At entry into our study, the newly developed lymphadenopathy was diagnosed as FL (CB/CC) by FNAC and DLBL (CB) by histopathology. The patient had stage I disease and was given local radiotherapy. After 1 year, later the patient experienced recurrence of lymphoma with a prompt response to CHOP chemotherapy.

Evaluation of adverse events

Two serious adverse events arose following surgical biopsy, including one patient who was hospitalized due to an infection and one patient who was reoperated due to local hemorrhage. Four non-serious adverse events were related to surgical biopsy including two patients who reported pain in the excision area requiring analgesics, one patient who suffered from a local infection, and one patient who reported a hematoma after biopsy. One non-serious adverse event was reported following FNAC: pain in the lymph node after FNA.

Discussion

The present study was undertaken to assess the diagnostic accuracy of FNAC versus surgical biopsy in a prospective cohort of patients with lymphadenopathy. Among patients who underwent FNA alone but were not included in the study ($n = 444$), 368 showed cytological and immunological features of reactive lymphadenopathy. Thus, the lymph node biopsy procedure in the majority of patients in the current series was selected on the basis of FNAC results showing suspected lymphoma (Figure 1). Our data cannot, therefore, contribute to the question of false negativity of FNAC. In 76 patients with lymphoma, a surgical biopsy was not performed due to several reasons such as: rapidly progressive disease with immediate need for treatment, no easily accessible lymph nodes, large tumor masses, high age, or a combination of of these factors. This fact highlights one distinct advantage of FNAC, that is, the possibility of a quick and accurate diagnosis. In patients at high risk for surgical complications, such as those with intra-abdominal, intrathoracic, orbital, thyroid, and intrapelvic lymphomas, FNAC may be the only means of diagnosis.[13,29,30] In Burkitt's and Burkitt variant lymphomas, where abdominal involvement is a common presenting feature, FNAC often leads to a diagnosis without resorting to laparotomy.[31]

In our study, both methods gave the same diagnosis in 74% of patients. The observed discrepancies confirm previous reports dealing with the pitfalls of FNAC.[10,13,21,32] Even though FNAC is accurate in the diagnosis of classical HL (cHL),[33] difficulties in differential diagnosis between cHL and ALCL may arise due to morphologic similarities in cytology.[34] In this case, the surgical biopsy provides information on the lymph node architecture and the distribution of neoplastic cells, necessary for definitive diagnosis. In addition, in cHL cases, the classification of various subtypes requires information on tissue architecture and hence surgical biopsy is required. In previous studies FNAC has been shown to have a high degree of accuracy ($>85\%$) in the diagnosis of cHL.[35–38] However, relatively poor results in subclassification have been reported, mainly due to limitations in the differentiation between the NS and MC types of cHL.[13,36,37] Other entities, such as T-cell rich B-cell lymphomas, HL lymphocyte predominance, T-cell lymphoma of AILD type, and other peripheral T-cell lymphomas can be difficult to diagnose by FNAC due to limitation in the evaluation of lymph node architecture and difficulties in identifying malignant cells.[24,39,40] Another major discrepancy concerns the grade classification and diagnosis of transformation in FL. In these cases, the differences may be due to FNA sampling error, because often both low-grade and transformed lymphoma are present in the same lymph node, and, in low-grade FL single follicles with higher numbers of centroblasts and increased proliferation may be present.[41] When highly represented in FNA material, these centroblasts may suggest transformation. Among the 10 cases with a major discordance, three (Nos. 1, 2 and 9) had an initial FNAC diagnosis of lymphoma,

while histopathology showed a reactive lymph node, probably due to the removal of another lymph node from the same site. Based on the clinical assessment, these patients were considered to have lymphoma and were thus treated accordingly.

Minor discrepancies in the classification of various subtypes of NHL depended partly on variable immunophenotyping results as obtained by immunostaining cytospins (FNAC), flow cytometry of lymph node suspensions and immunostaining of paraffin sections (histopathology). Most of the discrepancies in the immunophenotyping were related to the difficulty in the assessment of B-cell clonality by flow cytometry or immunohistochemistry on paraffin sections. These methods have, however, been improved during the recent years leading to significantly increased sensitivity and specificity. Overall, minor discrepancies as reported here did not have an impact on the treatment strategies, but it should be remembered that subtle diagnostic differences may influence treatment decisions in the future.

The use of FNA as a diagnostic tool in lymph node-based disease has historically been controversial.[42] Part of the reluctance in accepting FNA for the primary diagnosis of lymphoma originates from a time when recognition and classification of malignant lymphomas was difficult even in histological material.[43,44] Initially, FNAC was advocated as an adjunct to traditional surgical biopsy.[28,45,46] Recently, FNAC has become a more common practice in the primary diagnosis, subclassification, and management of patients with lymphoma.[13] However, the use of FNAC in various centers depends to a large extent on local traditions. The successful application of immunologic markers on material obtained by FNA has significantly promoted a wider acceptance of the use of FNAC in the final diagnosis of most non-Hodgkin lymphomas.[9,20,21,32] Based on a review of the literature,[9,10,13,21,22,35,36,45,47,48] the value and limitations of FNAC in the diagnosis of lymphomas should not be assessed in terms of cytohistological correlation alone with histology taken as the gold standard. FNAC is often used as a first line of investigation for screening cases with lymphadenopathy since this method is easy to perform as well as being rapid, and inexpensive. Here FNAC can help to differentiate between lymphoma, metastasis, nonhemopoietic neoplasms, specific infections such as tuberculosis lymphadenitis, and nonspecific reactive lymphadenitis.[36,48] In lymphoma patients, FNAC has a role in staging[13,49] and in the assessment of residual and/or recurrent disease[35,50] and may obviate the need for surgical biopsy in cases of lymphoma located in nonaccessible areas.[11,13,32,44,49,51]

An issue recently addressed by Dong et al.[52] is the availability of material for cytogenetic or molecular genetic analysis for diagnosis and of archival material for correlative scientific studies. When open biopsies are performed for diagnosis, there is always an archive of paraffin-embedded (and often frozen tissue) material that can be used for diagnostic and research purposes, molecular and genetic analyses, and additional immunophenotyping. In the present era of emerging technol-

ogies of genomics and proteomics, it is important to consider this limitation of FNAC. However, many laboratories, including ours, prepare additional smears, cytospin material and frozen-cell pellets that can be stored for future studies, and the suitability of this material for further studies including molecular genetic analysis has already been shown.[23,53,54] For academic centers with a commitment to diagnostic FNAC, consideration should be given to establishing banks of viably frozen cells. Today, immunophenotyping by either flow cytometry or immunocytochemistry is considered to be essential for the diagnosis of lymphoma. There is also growing evidence that the use of techniques focused on specific molecular abnormalities will have an increasing impact in the diagnostics of lymphomas in the future.[1]

Needle-core biopsies (NCB) should be considered as an alternative in lymphoma diagnostics.[55–57] This technique allows a minimal assessment of architecture in addition to immunostaining procedures. Image-guided NCB has been reported to be a quick, safe, and efficient alternative to excision biopsy. It may become the procedure of choice for histologic sampling in the absence of peripheral lymphadenopathy as suggested by Pappa et al.[57]

Based on the findings of the present study, we conclude that FNAC is an often accurate and safe method in the diagnosis of many subtypes of lympho-mas when the cytologic diagnosis is corroborated by immunocytochemistry. These findings are in good accordance with the results of a recent study confirming the accuracy of flow cytometry and cytomorphology of cells obtained by FNA of lymph nodes in lymphoma diagnostics.[58] It must be borne in mind that the present results have been obtained in a department with a long experience in FNAC.[12] The results may, therefore, not be entirely reproducible in other settings. A concern about the increasing use of FNAC for primary diagnosis and classification of lymphoma is the potential loss of archival tissue for complementary analyses, reclassification and research purposes. Freezing of cytospin material or cell pellets may, however, allow certain future analyses. It is also necessary to remember that some of the lymphoma entities (especially *T*-cell lymphomas) cannot be accurately diagnosed with FNAC. In addition, transformation events and composite lymphomas may be extremely difficult to diagnose without access to a surgical biopsy.

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